Original Article

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Profile of Multidrug Resistant Isolates from Paediatric Patients in a Tertiary Care Hospital, Puducherry

Microbiology Section

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ABSTRACT

Introduction: Nowadays, antimicrobial resistance is a challenge faced by physicians globally. Infections due to Multidrug Resistant Organisms (MDROs) is a significant problem in Paediatric Intensive Care Unit (PICU).

Aim: To identify the burden of antimicrobial resistance in hospital isolates from paediatric patients and elucidate the resistance pattern of MDROs.

Materials and Methods: A hospital based, prospective study was conducted for a period of two years. Samples from paediatric outpatients and inpatients were included and data were collected from those yielding growth on culture. Standard methods were followed for culture and antimicrobial susceptibility.

Results: A total of 5443 samples were received, from 3881 patients, including urine, blood, exudate, respiratory specimens, stool and body fluids. Total 561 bacteria were isolated from these samples with the majority being from urine 419 (74.7%) and blood 86 (15.3%). Of the 419 bacteria from urine, 238 (56.8%) and of the 86 isolates from blood, 24 (27.9%) were multidrug resistant, respectively. *Escherichia coli (E. coli)* was the most common pathogen as well as the most common MDRO followed by *Klebsiella* species. Multidrug resistant Gram negative bacteria were sensitive to amikacin (73-88%), piperacillin-tazobactam (82-100%) and imipenem (82-100%). *Enterococcus* spp. was the most resistant organism among Gram positive bacteria.

Conclusion: This study revealed a high prevalence of multidrug resistant pathogens among paediatric population. *E. coli, Klebsiella* spp. and *Enterococcus* spp. were the most common multidrug resistant pathogens, majority of them isolated from urine culture.

Keywords: Bacterial, Drug resistance, *Escherichia coli*, Urinary tract infections

INTRODUCTION

Nowadays, antimicrobial resistance is on the rise, creeping into the paediatric population too. Infections due to MDROs is a significant problem in paediatric ICU. In developing countries, MDROs causing neonatal infections are increasing and this has been attributed to the production of Extended Spectrum Beta Lactamases (ESBLs), Amp C β-lactamases, carbapenemases and aminoglycoside modifying enzymes [1]. Sepsis due to MDROs is a significant problem worldwide [1,2]. *Klebsiella pneumoniae* and *Staphylococcus aureus* have been identified as the most common cause for neonatal sepsis in India [3,4]. Prolonged hospitalisation, increased use of antimicrobials and vulnerability are some of the factors that favour neonatal colonisation with MDROs.

It has also been observed that MDROs causing Urinary Tract Infections (UTI) in the paediatric age group include mainly *E. coli* and *Klebsiella* spp. [5]. Infections due to Multidrug-Resistance (MDR) Gram negative organisms are on the rise globally [2,6-8].

The MDROs are not just limited to the hospital environment but have moved into the community as well, resulting in difficulty to treat infections. With the emergence of newer mechanisms of antimicrobial resistance among bacteria, it is mandatory for the laboratories and clinicians to keep a track on the trends in susceptibility pattern of the isolates for better management.

This study was done to know the burden of antimicrobial resistance among the isolates from paediatric patients, to know the profile of infections caused by MDROs and to study their resistance pattern.

MATERIALS AND METHODS

This was a hospital based, prospective study conducted during the period of September 2012 to June 2014 at the Department of Microbiology of a tertiary care centre in Puducherry, India. All the clinical specimen received from paediatric outpatients and inpatients were included in the study and data were collected from those yielding growth on culture. Repeat isolates from the same patients and from the same site/specimen were excluded. Ethical clearance was obtained from the Institute Ethics Committee for the study. Informed consent was obtained from parents. Standard methods were followed for sample processing, culture, and antibiotic susceptibility testing [9].

Blood cultures were done by inoculating 2-5 mL of blood, collected aseptically from median cubital vein into 20 mL of Brain Heart Infusion (BHI) broth. In case of neonates 1 mL of blood was inoculated into 5 mL of broth. It was incubated at 37°C for 18 hours and then subcultured onto Blood Agar (BA), Chocolate Agar (CA) and MacConkey (MAC) agar. Urine culture was done by standard loop technique, a semiquantitative method on Cystine Lactose Electrolyte Deficient (CLED) agar. Body fluids were centrifuged at 3000 rpm for 20-30 minutes and the sediment was inoculated onto BA, CA and MAC agar. Exudate samples were inoculated on BA and MAC. All plates were incubated at 37°C for 18 hours in 5-10% CO_2 jar. Those specimens which yielded growth was included in the analysis and the details were entered in Microsoft (MS) excel sheet. The antibiotic susceptibility pattern was analysed and expressed in percentages. All the clinically significant isolates were tested for susceptibility for various antibiotics (Hi-Media Laboratories Ltd, Mumbai, India) by Kirby-Bauer disc diffusion test as per CLSI 2012 guidelines [10]. Antibiotic panels tested for different organisms were as per CLSI guidelines. An organism was considered as ESBL producer when the zone size for ceftriaxone, cefotaxime and ceftazidime was ≤19 mm, 22 mm and 17 mm respectively [10].

For Quality Control of antibiotic susceptibility test, *Staphylococcus aureus* ATCC®25923™, *Escherichia coli* ATCC®25922™ and *Pseudomonas aeruginosa* ATCC®27853™ were used [10].

An organism was considered MDROs when it was resistant to more than or equal to three (≥3) drugs of different classes [11,12]. The common pathogens from various clinical samples and their susceptibility pattern were analysed. The relative occurrence of MDRO from various clinical specimens was also studied.

STATISTICAL ANALYSIS

The study was an observational study and the resistance patterns of organisms were calculated as percentages and proportions using MS Excel 2010 version.

RESULTS

A total of 5443 samples were received, from 3881 patients of paediatric age group, including urine (n=2826), blood (n=2208), exudates (n=52), respiratory specimens (n=141), stool (n=167) and body fluids (n=49) and 561 bacteria were isolated from these samples with the majority being from urine 419 (74.7%) and blood 86 (15.3%). The remaining bacteria were isolated from other specimen as shown in [Table/Fig-1]. Out of 561 isolates, 278 (49.5%) were MDRO. Out of the 419 bacteria isolated from urine, 238 (56.8%) were MDRO. Of the 86 isolates from blood, 24 (27.9%) were MDR. The details are as shown in [Table/Fig-1]. *E. coli* and *Klebsiella* spp. were the common pathogens isolated as shown in [Table/Fig-2]. Nearly 86% of the MDRO were isolated from urine culture as shown in [Table/Fig-3] and *E. coli* was the most common MDRO. The sensitivity pattern of these MDRO is as shown in [Table/Fig-4] and [Table/Fig-5]. Out of 561 culture positives, 39 were from neonates, 105 from one month to one year age group (infants) and 417 from 1-12 years age group.

DISCUSSION

Infections due to antibiotic resistant bacteria are of grave concern. Drug resistant bacteria are not only seen in hospital setting but also in the community. The increasing prevalence of drug resistant pathogens has become a global challenge, particularly among developing and under developed nations. Added to this are the poor infection control practices and abuse of antibiotics favouring the resistant pathogens in causing infections.

This study was undertaken to know the prevalence of MDROs in paediatric infections. Nearly 68% of the MDROs are from inpatients and 32% from outpatients suggesting the prevalence of resistant pathogens in community as well. In present study, urine and blood were the most commonly received clinical specimen and MDROs were also most commonly isolated from these specimens. About 561 isolates were obtained from blood, urine, exudate, respiratory, stool and body fluids, out of which 278 (49.5%) were MDROs. Nearly 57% of the isolates from urine and 28% of the isolates from blood were MDROs. Of the 278, 238 MDROs (86%) were isolated from urine. *E. coli*, *Klebsiella* spp. and *Enterococcus* spp. were the common MDROs in our study, where majority of these were isolated from urine samples.

Patients Registered	Urine			Blood Culture			Exudate			Respiratory samples			Stool culture			Body fluids		
	Total samples	No. of org*	MDR	Total samples	No. of org*	MDR	Total samples	No. of org*	MDR	Total samples	No. of org*	MDR	Total samples	No. of org*	MDR	Total samples	No. of org*	MDR
3881	2826	419	238	2208	86	24	52	36	14	141	14	1	167	5	1	49	1	0

[Table/Fig-1]: Summary of samples during study period (September 2012-June 2014).
*Number of organism

Organism	Urine	Blood culture	Exudate	Respiratory	Stool	Body fluids
Gram negative bacteria (n=436)						
E. coli (n=206)	198	3	4	-	1	-
Klebsiella spp. (n=72)	68	3	1	-	-	-
Acinetobacter spp. (n=30)	27	2	1	-	-	-
Citrobacter spp. (n=22)	16	6	-	-	-	-
Enterobacter spp. (n=14)	12	2				
*NFGNB (n=17)	12	5	-	-	-	-
Proteus spp. (n=22)	20	1	1	-	-	-
Providencia spp. (n=6)	6	-	-	-	-	-
Pseudomonas spp. (n=25)	12	9	4	-	-	-
S. typhi (n=10)		10				
S. paratyphi A (n=7)		6			1	
S. paratyphi B (n=2)		2				
Shigella spp. (n=3)	-	-	-	-	3	-
Gram positive cocci (n=125)						
*CONS (n=21)	2	18	1	-	-	-
Enterococcus spp. (n=47)	45	-	2	-	-	-
*MRCONS (n=7)	-	6	1	-	-	-
*MRSA (n=7)	-	-	7	-	-	-
S. aureus (n=19)	-	6	11	2	-	-
Streptococcus spp. (n=15)	1	5	3	6	-	-
*BHS (n=3)	-	1	-	2		
S. pneumoniae (n=6)	-	1	-	4	-	1

[Table/Fig-2]: Organisms isolated from various clinical specimen (n).
* NFGNB- Nonfermenting Gram Negative Bacilli; CONS- Coagulase Negative Staphylococci; MRCONS- Methicillin Resistant CONS; MRSA- Methicillin Resistant *Staphylococcus aureus*; BHS- Beta Haemolytic Streptococci.

Organism	Urine	Blood culture	Exudate	Respiratory	Stool	Body fluids
Gram negative bacteria (n=246)						
E. coli (n=150)	145	2	3	-	-	-
Klebsiella spp. (n=44)	41	2	1	-	-	-
Acinetobacter spp. (n=6)	6	--	-	-	-	-
Citrobacter spp. (n=12)	10	2	-	-	-	-
Enterobacter spp. (n=8)	7	1				
NFGNB (n=4)	2	2	-	-	-	-
Proteus spp. (n=13)	12	-	1	-	-	-
Providencia spp. (n=3)	3	-	-	-	-	-
Pseudomonas spp. (n=5)	4	1	-	-	-	-
Shigella spp. (n=1)	-	-	-	-	1	-
Gram postitve cocci (n=32)						
CONS (n=5)	-	5	-	-	-	-
Enterococcus spp. (n=10)	8	-	2	-	-	-
MRCONS (n=6)	-	5	1	-	-	-
MRSA (n=4)	-	-	4	-	-	-
S. aureus (n=3)	-	2	1	-	-	-
Streptococcus spp. (n=4)	-	2	1	1	-	-

[Table/Fig-3]: MDRO isolated from various clinical specimen.

Antibiotics	*E. coli* (n=150)	*Klebsiella* spp. (n= 44)	*Acinetobacter* spp. (n=6)	*Proteus* spp. (n= 13)	*Citrobacter* spp. (n=12)	*Pseudomonas* spp. (n=5)	*NFGNB (n=4)	*Enterobacter* spp. (n=8)	*Providencia* spp. (n=3)	*Shigella* spp. (n =1)
Amikacin	88.6	72.7	33.3	84.6	83.3	60	75	87.5	33.3	-
Ampicillin	2	4.5	16.6	15.4	0	-	25	0	-	0
Ampicillin-sulbactam	29.3	29.5	50	46.2	50	40	50	12.5	33.3	-
Cefepime	20.6	31.8	33.3	53.8	25	40	-	25	-	-
Ceftriaxone	6	22.7	33.3	69.2	16.6	-	0	12.5	33.3	0
Cefuroxime	5.3	9	0	23.1	0	-	25	0	-	-
Ciprofloxacin	4	11.3	-	23.1	16.6	60	75	-	0	-
Co-trimoxazole	9.3	22.7	50	15.4	25	-	50	0	33.3	0
Imipenem	92	81.8	83.3	100	100	80	75	100	100	0
Meropenem	86.6	52.2	66.6	61.5	33.3	40	-	75	100	-
Nitrofurantoin	79.3	50	0	15.4	50	0	0	62.5	33.3	-
Norfloxacin	13.3	50	33.3	61.5	25	0	25	50	66.6	-
Piperacillin-tazobactam	79.3	81.8	100	100	83.3	80	25	75	66.6	-
Piperacillin						80				-
Tobramycin	-	-	-	-	-	40	-	-	-	-

[Table/Fig-4]: Sensitivity pattern of MDR Gram negative bacilli (%).
*NFGNB- Non-fermenting Gram negative bacilli.

Studies from various parts of India had similar findings with *E. coli*, *Klebsiella* spp. and *Enterococcus* spp. being the most common uropathogens in children [3,12,13]. The susceptibility of enterobacteriaceae in a study was 96%, 51% and 58% towards imipenem, piperacillin and cefoperazone-sulbactam. *Enterococcus* spp. showed a resistance of 88%, 72% and 86% towards ciprofloxacin, amoxicillin and High Level Aminoglycoside Resistance (HLAR-G) respectively [3]. A study from Northern India showed that 42% of the Gram negative isolates were ESBL producers [12]. Similarly in the present study, 46% of the Gram negative organisms were ESBL producers where nearly 75% of them were *E. coli* isolated from urine.

Coagulase Negative Staphylococci (CONS), *E. coli* and *Klebsiella* spp. were the common MDR pathogens from blood culture. *Salmonella typhi and S. paratyphi A* were other significant isolates from blood culture. However, they were sensitive to the antibiotics tested. *Enterobacter* spp. and *Klebsiella pneumoniae* were common Gram negative pathogens and CONS and *S. aureus* were common Gram positive pathogens causing neonatal septicemia in various studies across India [14-17]. These studies showed that Gram negative bacilli were sensitive to ciprofloxacin and amikacin and were highly resistant to third generation cephalosporins. Studies have shown that septicaemia due to drug resistant Gram negative bacilli are important cause for neonatal mortality [18,19].

A study conducted on septicaemic neonates in Bijapur, Karnataka showed that *K. pneumoniae* was highly resistant to ciprofloxacin (71%), amikacin (67%) and aztreonam (92%) [1].

A study in Taiwan showed that nearly 20% of the bacteremia among neonates in Neonatal ICU (NICU) was caused by MDR Gram negative bacilli. *K. pneumoniae* was the most common causative agent followed by *E. coli*. Prior broad spectrum antibiotic therapy and underlying renal disease were some of the risk factors

Antibiotics	*Enterococcus* spp. (n=10)	#MRCONS (n=6)	#CONS (n=5)	#MRSA (n=4)	*Strepto coccus* spp. (n=4)	*S. aureus* (n=3)
Ampicillin	73					
Cefoxitin	-	0	100	0	0	100
Ciproflox	0	50	80	25	25	33.3
Clindamycin	-	83.3	100	100	25	0
Chloramphenicol	90	83.3	80	50	75	100
Co-trimoxazole	-	33.3	40	50	50	0
Erythromycin	-	33.3	0	25	0	0
Gentamicin	40 (HLG)*	66.6	80	25	75	100
Linezolid	100	100	100	100	-	100
Penicillin	-	0	0	0	25	0
Teicoplanin	100	83.3	100	50	-	100
Tetracycline	40	50	80	25	25	
Vancomycin	60	83.3	100	-	-	-
Nitrofurantoin	50	-	-			

[Table/Fig-5]: Sensitivity pattern of MDR Gram positive cocci (%).
*HLG- High Level Gentamicin.
#CONS- Coagulase Negative Staphylococci; MRCONS – Methicillin Resistant CONS ; MRSA – Methicillin Resistant Staphylococcus aureus.

associated [20]. *Acinetobacter* spp. and *K. pneumoniae* were the most common MDR pathogens in PICU in a study in Pakistan. These organisms showed high resistance to ceftriaxone, amikacin and piperacillin-tazobactam [5].

A study from Karnataka states that MDR *Acinetobacter* spp. were also potential pathogens causing septicaemia in neonates [21]. However, in our study we did not encounter MDR *Acinetobacter* spp. from blood though few were isolated from urine.

A study on neonatal septicaemia in a tertiary care centre in Maharashtra showed that *K. pneumoniae* and *S. aureus* were the common pathogens and that 28% of *K. pneumoniae* and *E .coli* were ESBL producers. Multidrug resistant pattern was observed in their study and ciprofloxacin and aminoglycosides were found to be useful drugs [4].

The MDR Gram negative bacilli in our study showed good sensitivity to amikacin (73-88%), pipercillin-tazobactam (82-100%) and imipenem (82-100%). Sensitivity to other antimicrobials was poor (<30%). Among the MDR Gram positive cocci, Enterococci were the significant one which showed high sensitivity to ampicillin (73%), chloramphenicol (90%), linezolid (100%), teicoplanin (100%) though 40% of the Enterococci were Vancomycin Resistant *Enterococcus* (VRE).

Studies have shown that in infections due to MDR bacteria, an appropriate antibiotic combination therapy needs to be prescribed based on the local antibiogram, both for empirical as well as therapeutic purpose [22].

LIMITATION

Though the study was done prospectively, clinical correlation and follow up of patients were not feasible. Hence, only laboratory data were analysed. Molecular analysis for resistant isolates has been sent to AIIMS, New Delhi, the results of which are awaited.

CONCLUSION

This study reveals significant prevalence of MDR pathogens among paediatric population. *E. coli, Klebsiella* spp. and *Enterococcus* spp. were the common MDROs encountered in the study, mostly isolated from urine culture. MDROs are present among outpatients as well, warranting judicious use of antibiotics and adequate infection control measures to prevent spread of these potential pathogens.

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